Ex vivo whole embryonic kidney culture: a novel method for research in development, regeneration and transplantation.

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Public Summary:
PURPOSE: Whole metanephric organ culture represents a novel investigatory approach with potential application in many aspects of research in kidney regeneration and transplantation. We report the current status of embryonic kidney culture, discussing issues such as the appropriate culture conditions and methods, histological results, values of and limitations to the different techniques used today. To optimize this system in vitro for the benefit of future studies we focused our efforts on evaluating and developing a new durable 3-dimensional organ culture system using a uniquely modified approach. MATERIALS AND METHODS: Metanephric kidneys were microdissected from the embryos of timed pregnant WT C57BL/C6 mice on days 12 to 16 of gestation (embryonic days 12 to 16). Novel perfusion channels were created in the harvested embryonic kidneys before placing them in culture. Embryonic kidneys were placed on a 0.4 microm pore size Transwell membrane, cultured in base medium at a medium gas interphase and incubated at 37C with fully humidified 5% CO2. Histological and immunocytochemical analysis was performed to evaluate for signs of necrosis, and the structural integrity and functionality of organs during culture. RESULTS: We confirmed histologically that our organ culture system was capable of maintaining normal kidney structures significantly longer (mean 10 days) than previously reported standard protocols. Condensation and aggregation of the metanephric mesenchyma at the tips of the ureteral bud were observed, including the formation of well developed nephrons and glomeruli without evidence of necrosis. Organ maturation occurred in a developmentally appropriate centrifugal pattern and the expression of key regulatory factors was demonstrated. CONCLUSIONS: Our in vitro model replicates closely the in vivo processes involved in normal kidney development. We also present what is to our knowledge the first demonstration of a durable 3-dimensional kidney culture system reported in the literature. This system may represent an uncomplicated method for in vitro kidney culture that we hope will serve as an effective adjunct to research focused on signaling pathways, development and regeneration as applied to the kidney.

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